IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of:

Bruce Joseph ROSER

Serial No.: 09/888,734

Filing Date: 25 June 2001

For: DRIED BLOOD FACTOR COMPOSITION COMPRISING TREHALOSE

Examiner: Francisco Chandler Prats

Group Art Unit: 1651

DECLARATION OF EDWARD G. D. TUDDENHAM

UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Edward G D Tuddenham, declare as follows:

1. I am Professor of Haemostasis at the Imperial College Faculty of Medicine, London, UK. I am familiar with the terminology used in the field of blood-derived factors, and with the nature of Factor VIII. I have been practicing in this field for thirty years. A copy of my *curriculum vitae* is attached.

2. I have reviewed the above-referenced application in its entirety. This application nowhere describes the treatment of Factor VIII with thrombin or any other protease to effect its activation. At no location is "activated Factor VIII" referred to. If such treatment had been performed, the "Factor VIII" would be referred to as "activated Factor VIII" or some additional

description would be given to indicate that the Factor VIII as it would be found in plasma, or as it would be recombinantly produced using the gene encoding that found in plasma, had been treated with a protease. I have been told that the record in this application indicates that the US Patent Office ("the Office) agrees with the foregoing, and that the Office understands the claims as presently presented are directed to methods to prepare a stable, dried composition of "native" Factor VIII, i.e., they refer to a Factor VIII other than that "activated" by thrombin treatment.

3. I have reviewed the Office action mailed 1 September 2004 and note the comments on page 6 stating that "the proteins (i.e., native Factor VIII and activated Factor VIII) possess numerous virtually identical amino acid sequences." However, substantial portions of the native form are missing from activated Factor VIII. Factor VIII is a heterodimer of a heterogeneous 90 - 210 kD heavy chain, having a 90 kD constant region and a variable region of up to 120 kD, and a light chain of 80 kD. This is the case whether the Factor VIII is in the form that circulates in the plasma, or the commercial form obtained from plasma, or the commercial form obtained by recombinant DNA techniques. However, *activated* Factor VIII is a hetero*trimer* containing only a portion of the native Factor VIII light chain (73 kD) and two fragments of the constant region of the heavy chain of 50 kD and 43 kD. Although some amino acid sequences are retained, they are rearranged and a very large portion (amounting to an average of 124 kD (out of a total heterodimeric mass of 290 kD)) is missing. Thus, almost half of the amino acid sequences of native Factor VIII are missing from activated Factor VIII.

4. Page 6 also states "at the very least, Curtis establishes generally that Factor VIII has a therapeutic utility that can be preserved upon freeze-drying in the presence of trehalose." This is not entirely accurate. Curtis concerns only preservation of activated Factor VIII, which activated Factor VIII does have therapeutic activity, while native Factor VIII does not (absent

activation by protease). The behavior of the already active molecule is not informative with respect to the behavior of the circulating heterodimeric form, which represents native Factor VIII, since that form is not active at all. Indeed it is referred to in the literature as the 'procofactor', which is analogous to the zymogen of the protease factor thrombin called prothrombin. Thus, even if Curtis showed that the activated form of Factor VIII, which itself has cofactor activity, could be stably preserved in the presence of trehalose, there is no scientific basis to extrapolate this to the native Factor VIII, which lacks such cofactor activity until proteolytically cleaved by thrombin. It simply does not follow. Great care is taken in the literature and in practice to distinguish between the inactive pro- forms of clotting factors and their activated forms as these have highly different properties. For example the activated forms are thrombogenic and have shortened half-lives *in vivo*.

5. In my opinion, the activated form of Factor VIII and the native form of Factor VIII (as a circulating heterodimer) are sufficiently different that the physical behavior of one is not predictive of the physical behavior of the other. Not only is almost half of the amino acid sequence missing in the activated form; the arrangement of the remaining peptides is different. Instead of a heterodimer with a heterogeneous heavy chain of 90-210 kD and a light chain of 80 kD, the activated form is a trimer that is not heterogeneous and comprises two segments of 50 and 43 kD, as well as a 73 kD monomer. In view of the heterogeneity of the native Factor VIII and in view of its markedly different structure from activated Factor VIII, the behavior of these materials would be expected to be very different rather than similar.

6. I have also reviewed U.S. Patent 5,364,756 to Livesey, *et al.* I have noted that claim 17, dependent on claim 1, specifies the material subjected to the process of claim 1 as Factor VIII. Claim 1 is directed to a method for preserving a suspension of biological material,

which comprises preparing a cryosolution using a suspension of biological material. With respect I submit that to chemists, pharmacologists and biologists a suspension means particulate matter held dispersed in some fluid, not a molecular substance dissolved in some aqueous solution. I am familiar with the solubility of characteristics of Factor VIII and can verify that a suspension of Factor VIII as condensed perhaps crystalline particles could reasonably be prepared only by use of extremely high concentrations that are not realistically contemplated or by denaturing the protein. The suspensions exemplified in the Livesey patent are of insoluble materials such as cells or viruses. The description of Livesey is inappropriate to Factor VIII, which is alluded to, inexplicably, in a 'shopping list' of materials that reasonably relate to Livesey's process in column 4, lines 57-64. A skilled practitioner of the art, familiar with the characteristics of Factor VIII, would understand that the inclusion of Factor VIII in such a list in this context is clearly an error or an optimistic attempt to be all encompassing and hence over inclusive.

I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements are made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Executed at London, United Kingdom, on 23rd November 2004.
(city) (state) (day)

E. G. D. Tuddenham
(name)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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Bruce Joseph ROSER	Group Art Unit: 1651
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Declaration of Sam L Helgerson

under 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Sam L Helgerson, declare as follows:

1. I am the same Sam L. Helgerson who is a co-inventor for US patent No.5,824,780 (Curtis *et al*). I have been with the Baxter Healthcare Corporation since 1991 and have worked during that time as a Research Scientist on projects to develop protein-based biotherapeutic products. I am now Senior Research Director in the Baxter BioScience R&D Group.

2. I have reviewed the Office Action mailed September 1, 2004 and I note the comments on page 6 stating that "the proteins (i.e., native Factor VIII and activated Factor VIII) possess numerous virtually identical amino acid sequences." However, although Factor VIIIa shares all of its amino acid sequence with Factor VIII, the two are in fact significantly different from one another in several key structural and functional aspects a was stated in the Background section of Patent 5,824,780: "Recent advances in the isolation of Factor VIII and the molecular cloning of the Factor VIII gene have revealed that the primary structure of Factor VIII contains several distinct types of structural domains. There are three A domains, A1, A2, and A3 each of approximately 350 amino acids, a unique region of about 980 amino acids called the B domain, and a carboxyl-terminal region of about 300 amino acids

called the C1-C2 domain. These domains are arranged in human Factor VIII in the order of A1-A2-B-A3C1-C2 (Vehar et al. Nature 312:327 to 342, 1984). Treatment of procoagulant protein Factor VIII with thrombin results in an increase in coagulant activity, which is associated with the formation of an activated form of Factor VIII. Previous attempts to isolate and characterize the activated form of human Factor VIII have been unsuccessful because the activity of this form rapidly decays. The activation of Factor VIII by thrombin has been shown to coincide with cleavage of the polypeptide chain at residue position 372 between the A1 and A2 domains, at position 740 between the A2 and B domains, at unidentified positions within the B domain, and at position 1689 between the B and A3-C1-C2 domains. The active Factor VIII complex then forms as a heterotrimer composed of the A1, A2, and A3-C1-C2 subunits." Hence, both the gross molecular sizes and the intramolecular subunit interactions of the two proteins are very different. Importantly, the activation of FVIII to FVIIIa is required in order to achieve the fully functional properties required for blood coagulation activity.

3. Page 6 of the office action also states that "at the very least, Curtis establishes generally that Factor VIII has a therapeutic utility that can be preserved upon freeze-drying in the presence of trehalose." I believe that this may overstate the utility of our work. In the Curtis *et al* patent (on which, as noted above, I was a co-inventor), we focused the disclosure to methods and formulations for stabilising the final activated Factor VIII protein, in other words Factor VIIIa. The protein structure of FVIIIa required for functional blood coagulation activity is highly dependent on specific intramolecular subunit interactions that are unique to FVIIIa in comparison to FVIII. In particular, these subunit interactions are very labile and must be stabilized in order for the desired activity to be maintained. Our work with protein stabilizing agents, i.e., human serum albumin, sucrose, and trehalose, was aimed specifically at solving this problem. We did not seek to extend the teaching of the patent disclosure to unactivated Factor VIII, in other words simply "Factor VIII". I believe that a person working in this field would have duly noted this and would not have assumed that the patent was teaching methods and formulations for stabilising Factor VIII. Because the two protein forms are so different from one another, the attributes of, uses of, and techniques involving one may not simply extrapolated to the other.

I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements are made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Executed at Fremont, CA, on 30 November 2004.

(city) (state) (day)

Sam S. Nelgerson
(name)